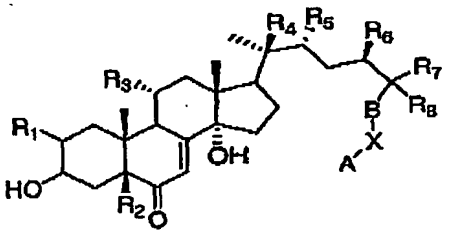
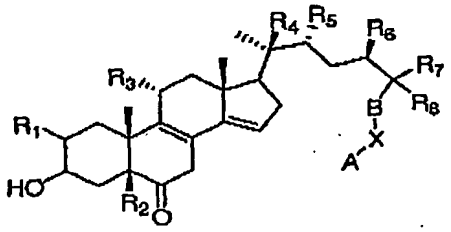
Claims

1. An ecdysteroid derivative wherein a fluorescent moiety is attached to an ecdysteroid moiety by derivatisation of a hydroxyl group on the alkyl side chain of the ecdysteroid moiety, wherein the derivative is capable of binding to an ecdysone receptor or ligand binding domain thereof.

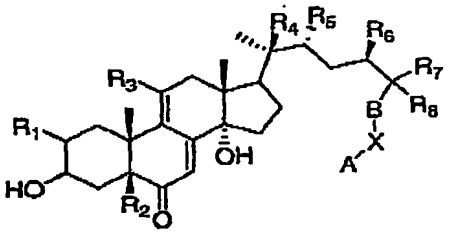
2. An ecdysteroid derivative according to claim 1 wherein the derivative has general structure 1a, 2a, or 3a;



1a



2a



3a

wherein B is CH_2O, CH_2S, CH_2NH, O, S, or NH; X is a linking group; A is a fluorescent moiety; R^1-R^5 are independently selected from H, alkyl, haloalkyl, OH, or halogen, R^7- R^8 are independently selected from H, alkyl, haloalkyl, OH, or halogen or R^7 and R^8 together are $=CH_2$; R^6 is selected from H, OH, alkyl, $=CH_2$ or halogen, or R^6 together with R^7 is a double bond.

3. A derivative according to claim 2 wherein the alkyl groups are C1 to C20, more preferably, for R^1 and R^2 the alkyl group is C1 to C5.

4. A derivative according to claim 2 wherein R^1, and R^5 are OH; R^2 is H or OH; R^3 is H; R^4 is H or OH; R^6 is selected from H, OH, CH_3, CH_3CH_2, or $(CH_3)_2$ CH; R^7 and R^8 are

independently selected from H, OH, CH_3, or R^7 and R^8 together can be = CH_2, R^6 together with R^7 can be a double bond.

5. A derivative according to any one of claims 2 to 4 wherein X is selected from the group consisting of C(O)NH, C(S)NH, SO_2 and C(O).

6. A derivative according to any one of claims 2 to 5 wherein B is CH_2O or O.

7. A derivative according to any one of claims 2 to 6 wherein the fluorescent moiety is selected from the group consisting of unsubstituted and substituted fluorescein moieties, unsubstituted and substituted dansyl moieties, and unsubstituted and substituted coumarin moieties.

8. A derivative according to any one of claims 2 to 7 wherein the derivative is of general structure 1a wherein R^1 and R^5 are OH, R^3 is H, R^7 is CH_3, and B is CH_2O and X is selected from the group consisting of C(O)NH, C(S)NH, SO_2 and C(O).

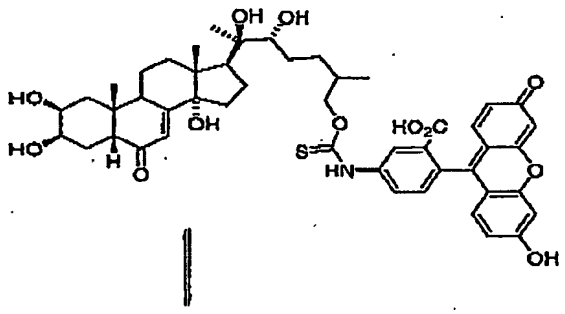
9. A derivative according to claim 8 wherein R^2, R^4, and R^8 are independently selected from H, alkyl, OH, or halogen; R^6 is selected from H, OH, alkyl, $=CH_2$ or halogen.

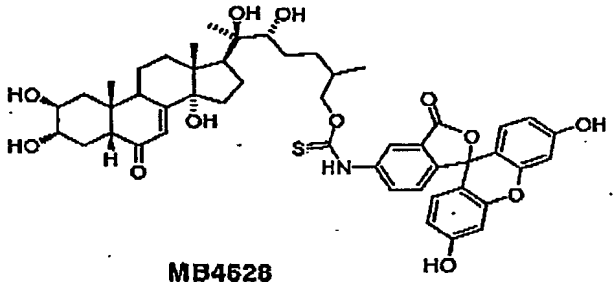
10. A derivative according to claim 9 wherein R^2 is H, R^4 is OH, R^6 is H and R^8 is H.

11. A derivative according to any one of claims 2 to 7 wherein R^1, R^4 and R^5 are OH, R^2 is H or OH, R^3 is H, R^6 is H or CH_3, and R^7 and R^8 are H or CH_3.

12. A derivative according to any one of claims 1 to 11 wherein the ecdysteroid moiety is selected from the group consisting of inokosterone, 26-hydroxyecdysone, 20,26-dihydroxyecdysone, makisterone B, amarasterone A, amarasterone B, ajugasterone B, sidasterone A, sidasterone B, 26-hydroxy-polypodine B, 20-hydroxyecdysone, makisterone A, polypodine B and rapisterone D.

13. A derivative according to any one of claims 1 to 12 wherein the derivative is selected from the group consisting of:





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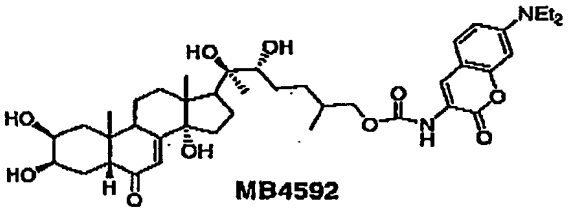

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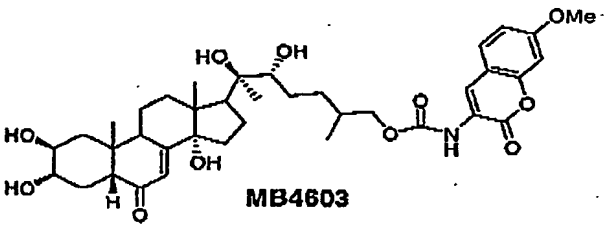

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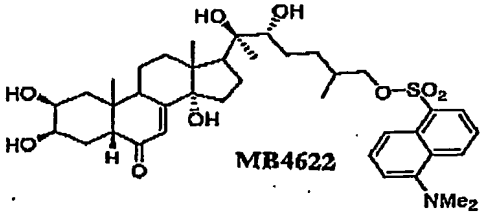

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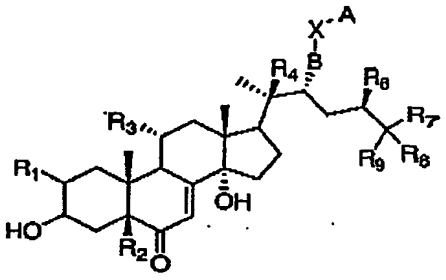
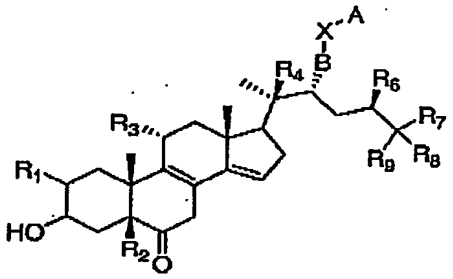
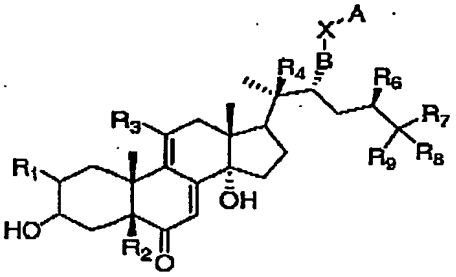
14. An ecdysteroid derivative according to claim 1 wherein the derivative has general structure 1b, 2b, or 3b;



1b



2b



3b

wherein B is CH_2O, CH_2S, CH_2NH, O, S, or NH; X is a linking group; A is a fluorescent moiety; R^1-R^4, R^7-R^9 are independently selected from H, alkyl, haloalkyl, OH, or halogen; R^6 is selected from H, OH, alkyl, $=CH_2$ or halogen.

15. A derivative according to claim 14 wherein the alkyl groups are C1 to C20, more preferably, for R^1 and R^2 the alkyl group is C1 to C5.

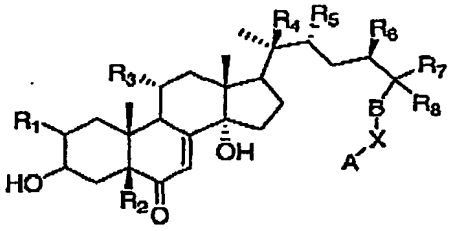
16. A derivative according to any one of claims 14 to 15 wherein X is selected from the group consisting of C(O)NH, C(S)NH, SO_2, and C(O).

17. A derivative according to any one of claims 14 to 16 wherein the fluorescent moiety is selected from the group consisting of unsubstituted and substituted fluorescein moieties, unsubstituted and substituted dansyl moieties, and unsubstituted and substituted coumarin moieties.

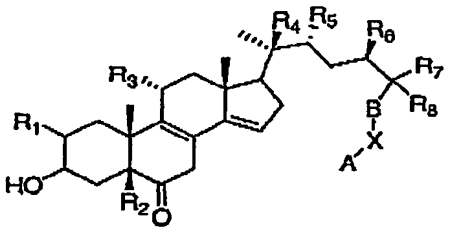
18. A derivative according to any one of claims 14 to 17 wherein R^1 and R^4 are OH; R^2 and R^3 are independently selected from H or OH; R^6 is selected from H or CH_3, and R^7, R^8 and R^9 are independently selected from the group H, OH, CH_3, F, and I.

19. A derivative according to any one of claims 14 to 18 wherein the ecdysteroid moiety is selected from the group consisting of ponasterone A, 20-hydroxyecdysone, muristerone A, makisterone A, polypodine B, rapisterone D, 2β,3β,20*R*,22*R*-tetrahydroxy-25-fluoro-5β-cholest-8,14-dien-6-one, 5-deoxykaladasterone, 26-iodoponasterone A, and 25-fluoroponasterone A.

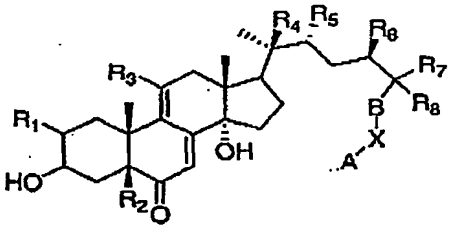
20. A compound selected from the group of compounds consisting of general structures **1a**, **2a**, and **3a** which interact with an ecdysone receptor or ligand binding domain (LBD) thereof;



1a



2a



3a

wherein B is CH_2O, CH_2S, CH_2NH, O, S, or NH; X is a linking group; A is a fluorescent moiety; R^1-R^5 are independently selected from H, alkyl, haloalkyl, OH, or halogen, R^7- R^8 are independently selected from H, alkyl, haloalkyl, OH, or halogen or R^7 and R^8 together are $=CH_2$; R^6 is selected from H, OH, alkyl, $=CH_2$ or halogen, or R^6 together with R^7 is a double bond.

21. A compound according to claim 20 wherein the alkyl groups are C1 to C20, more preferably, for R^1 and R^2 the alkyl group is C1 to C5.

22. A compound according to claim 20 or 21 wherein R^1, and R^5 are OH; R^2 is H or OH; R^3 is H; R^4 is H or OH; R^6 is selected from H, OH, CH_3, CH_3CH_2, or $(CH_3)_2CH$; R^7 and R^8 are independently selected from H, OH, CH_3, or R^7 and R^8 together can be $=CH_2$, R^6 together with R^7 can be a double bond.

23. A compound according to any one of claims 20 to 22 wherein X is selected from the group consisting of C(O)NH, C(S)NH, SO_2 and C(O).

24. A compound according to any one of claims 20 to 23 wherein B is CH_2O or O.

25. A compound according to any one of claims 20 to 24 wherein the fluorescent moiety is selected from the group consisting of unsubstituted and substituted fluorescein moieties, unsubstituted and substituted dansyl moieties, and unsubstituted and substituted coumarin moieties..

26. A compound according to any one of claims 20 to 25 wherein the compound is of general structure 1a wherein R^1 and R^5 are OH, R^3 is H, R^7 is CH_3, and B is CH_2O and X is selected from the group consisting of C(O)NH, C(S)NH, SO_2 and C(O).

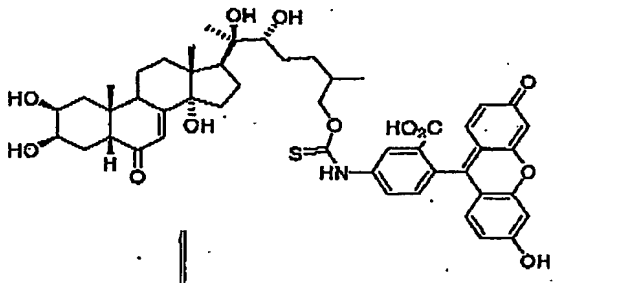
27. A compound according to claim 26 wherein R^2, R^4, and R^8 are independently selected from H, alkyl, OH, or halogen; R^6 is selected from H, OH, alkyl, $=CH_2$ or halogen.

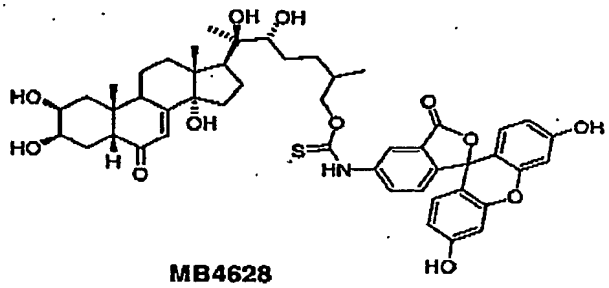
28. A compound according to claim 27 wherein R^2 is H, R^4 is OH, R^6 is H and R^8 is H.

29. A compound according to any one of claims 20 to 28 wherein R^1, R^4 and R^5 are OH, R^2 is H or OH, R^3 is H, R^6 is H or CH_3, and R^7 and R^8 are CH_3.

30. A compound according to any one of claims 20 to 29 wherein the fluorescent moiety is attached by derivatisation to an ecdysteroid selected from the group consisting of inokosterone, 26-hydroxyecdysone, 20,26-dihydroxyecdysone, makisterone B, amarasterone A, amarasterone B, ajugasterone B, sidasterone A, sidasterone B, 26-hydroxy-polypodine B, 20-hydroxyecdysone, makisterone A, polypodine B and rapisterone D.

31. A compound according to any one of claims 20 to 30 wherein the compound is selected from the group consisting of:





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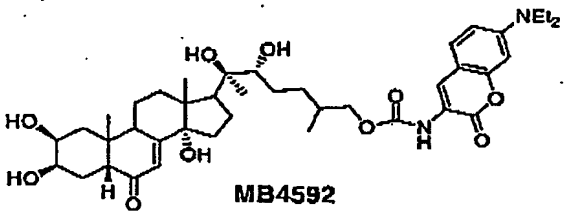

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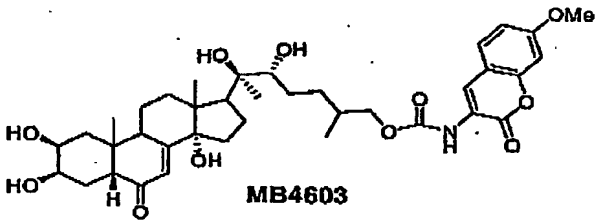

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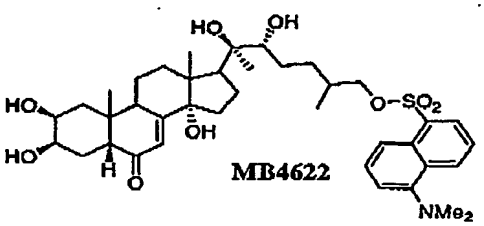

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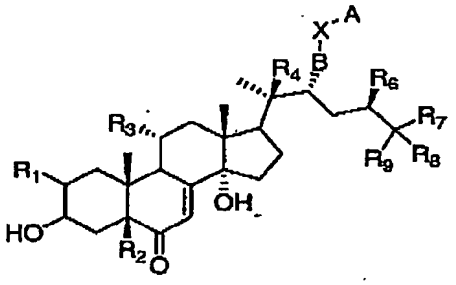
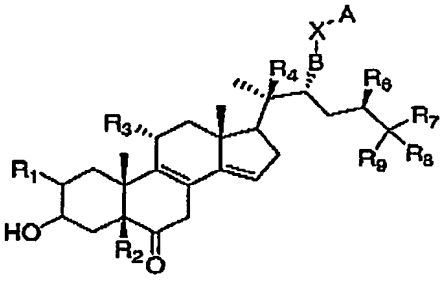
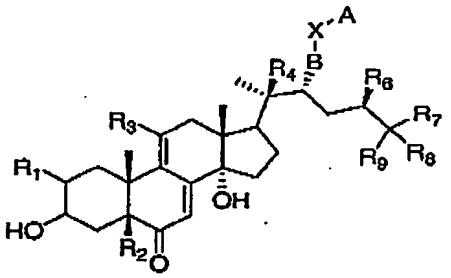
32. A compound selected from the group of compounds consisting of general structures 1b, 2b, and 3b which interact with an ecdysone receptor or ligand binding domain (LBD) thereof;



1b



2b



3b

wherein B is CH_2O, CH_2S, CH_2NH, O, S, or NH; X is a linking group; A is a fluorescent moiety; R^1-R^4, R^7-R^9 are independently selected from H, alkyl, haloalkyl, OH, or halogen; R^6 is selected from H, OH, alkyl, =CHI_2 or halogen.

33. A compound according to claim 32 wherein the alkyl groups are C1 to C20, more preferably, for R^1 and R^2 the alkyl group is C1 to C5.

34. A compound according to any one of claims 32 to 33 wherein X is selected from the group consisting of C(O)NH, C(S)NH, SO_2, and C(O).

35. A compound according to any one of claims 32 to 34 wherein the fluorescent moiety is selected from the group consisting of unsubstituted and substituted fluorescein moieties, unsubstituted and substituted dansyl moieties, and unsubstituted and substituted coumarin moieties.

36. A compound according to any one of claims 32 to 35 wherein R^1 and R^4 are OH; R^2 and R^3 are independently selected from H or OH; R^6 is selected from H or CH_3, and R^7, R^8 and R^9 are independently selected from the group H, OH, CH_3, F, and I.

37. A compound according to any one of claims 32 to 36 wherein the fluorescent moiety is attached by derivatisation to an ecdysteroid is selected from the group consisting of ponasterone A, 20-hydroxyecdysone, muristerone A, makisterone A, polypodine B, rapisterone D, 2β,3β,20*R*,22*R*-tetrahydroxy-25-fluoro-5β-cholest-8,14-dien-6-one, 5-deoxykaladasterone, 26-iodoponasterone A, and 25-fluoroponasterone A.

38. A compound which is an ecdysteroid mimic wherein the compound comprises a non-ecdysteroid moiety that interacts with an ecdysone receptor or ligand binding domain thereof, and wherein the compound further comprises a fluorescent moiety.

39. A compound according to claim 38 wherein the compound comprises a substituted or unsubstituted dibenzoyl hydrazine moiety that interacts with an ecdysone receptor or ligand binding domain thereof, and wherein the compound further comprises a fluorescent moiety attached through a phenyl ring substitutent or a nitrogen atom in the dibenzoyl hydrazine moiety.

40. A method for screening a candidate compound for its ability to interact with an ecdysone receptor or ligand binding domain (LBD) thereof in a competitive inhibition format, the method comprising the steps of:

(a) incubating with an ecdysone receptor or LBD thereof, a candidate compound and the derivative according to any one of claims 1 to 19; and

(b) measuring the extent of binding of the derivative according to any one of claims 1 to 19 to the ecdysone receptor or LBD thereof.

41. A method for screening a candidate compound for its ability to interact with an ecdysone receptor or ligand binding domain (LBD) thereof in a competitive inhibition format, the method comprising the steps of:

(a) incubating with an ecdysone receptor or LBD thereof, a candidate compound and the compound according to any one of claims 20 to 37; and

(b) measuring the extent of binding of the compound according to any one of claims 20 to 37 to the ecdysone receptor or LBD thereof.

42. A method for screening a candidate compound for its ability to interact with an ecdysone receptor or ligand binding domain (LBD) thereof in a competitive inhibition format, the method comprising the steps of:

(a) incubating with an ecdysone receptor or LBD thereof, a candidate compound and the derivative according to any one of claims 38 or 39; and

(b) measuring the extent of binding of the derivative according to any one of claims 38 or 39 to the ecdysone receptor or LBD thereof.

43. A method according to any one of claims 40-42 wherein the competitive inhibition format is a fluorescence polarization assay.

44. A method according to any one of claims 40 to 43, wherein the assay is conducted in a microtitre plate well.

45. An insecticidal compound identified by the assay according to any one of claims 40 to 44.

46. An effector compound for ecdysone receptor gene switches, the compound identified by the assay according to any one of claims 40 to 44.